ImmunoTools *special* Award 2014

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The role of Notch in the development of drug resistance and stemness maintenance in multiple myeloma.

Multiple myeloma (MM) is a neoplastic plasma cell disorder. MM therapy is rarely curative, and a median survival is about 3-4 years after diagnosis. Indeed, despite the last therapeutic advances, MM is still incurable because of chemotherapeutic resistance mainly resulting from MM cells interaction with the bone marrow (BM) milieu. BM represent a safe haven for MM bulk and stem cell population. MM stem cells (MMSCs) sustain MM maintenance and disease burden and are responsible of pharmacological resistance. As a matter of fact, MM relapse is most likely due to MMSCs surviving to chemotherapy.

Eradicating MMSCs could greatly increase MM patients survival. Therefore this project aims to precisely characterize MMSCs.

Up to now, results from several studies on MMSCs characterization provided discordant conclusions concerning the phenotype displayed by MM cells with clonogenic potential, possibly due to a high level of heterogeneity of MMSCs sub-populations. Specifically, the following immunophenotypes have been reported to share stemness properties: $CD138^{-}$ $CD20^{+}$, $CD38^{++}CD45^{-}$, $CD138^{-}CD19^{+}CD27^{+}$, $CD34^{+}CD38^{+}$.

To contribute to MMSCs characterization, we will isolate cells displaying these phenotypes from MM patients' peripheral blood or BM and perform a clonogenicity assay to evaluate the stemness potential of the different sub-populations.

The MMSC population identified in this way will be further studied to understand:

- the mechanisms underlying BM stromal cells (BMSC) ability to increase the number of resilient CSCs;
- if a reciprocal stimulation is active between CSCs and BMSCs.

At this purpose, different co-culture systems will be set up including MM cell lines and primary cells and BMSC lines as HS-5 or HS-27. An ELISA assay will be helpful to assess if MM cells may induce BMSCs to secrete TNF-α, hIL6, hIGF1 and hVEGF.

A further step will specifically address the role of the Notch pathway, recently proposed as a promising therapeutic target in MM, in MSC maintenance. Indeed, Notch signaling is deregulated in MM cells, influences MM cell proliferation and survival and the interaction with the BM, resulting in increased resistance to chemotherapeutics.

In order to evaluate the role of Notch in CSCs maintenance we will use a specific shRNA to silence the expression of Notch1 receptor, which has been reported to be upregulated from MGUS (a benign form preceding MM) to myeloma.

Notch1-silenced MM cells will be characterized by flow cytometry to determine if Notch depletion causes a decrease in MMSC population. To this, we will use the panel of antibodies previously identified.

Moreover we will assess the effects of Notch1 silencing on biological feature relevant in the biology of MM cells and MMSCs: cells growth, survival and chemotaxis.
An apoptosis assay will be performed by using Annexin V and propidium iodide on MM cells transfected with the anti-Notch1 shRNA compared to the control cells (scrambled siRNA).
The effects of Notch on MM cells migration will be assess analyzing the ability of MM cells to answer to the chemotactic stimulus due to recombinant hMIP-1α, hRANTES, hMCP2, hSDF1a and hIL6 after Notch1 depletion.

Direct interaction between MM cells and BM cells along with secreted growth factors activate pleiotropic signals that promote growth, survival, drug resistance and migration of MM cells. We hypothesize that Notch1 activation in MM cells and BMSC, actively participates in this process. Therefore we will assess if Notch withdrawal in MM cells may alter their adhesion to ECM or to stromal cells and affect relevant biological parameters, including stemness maintenance, drug resistance, apoptosis, proliferation and stromal cells-mediated production of paracrine factors relevant to MM.
To address this issue, co-cultures of silenced MM cell lines/primary cultures and BMSCs will be maintained up to 6 days and treated with Bortezomib, a proteasome inhibitor currently used in MM therapy. Apoptosis assay will be performed to evaluate the role of Notch in BMSC-dependent drug resistance. Flow cytometry and quantitative RT-PCR (qRT-PCR) will allow to assess variations in the expression of factors inducing MM cell proliferation, apoptosis resistance and stemness, i.e. IL-6, SDF1α, IGF-1, VEGF, TNF-α, bFGF, OPG/RANKL ratio, MIP-1α, MIP-1β and the stemness markers identified in the previous steps.
The relevance of this project stems from the need of identifying an alternative therapy which may address the issue of drug resistance and patients relapse. We aims to explore the possibility to use a molecularly targeted approach selectively directed to Notch1 due to the high toxicity of the treatments with GSIs. These Notch inhibitors indeed affects the activation of all the 4 Notch isoforms, including Notch2 which is responsible of gut toxicity. This work may provide the rational for an effective and safer Notch-directed approach to contrast MM patients relapse and drug resistance, improving the response to standard treatments, providing a valuable option for patients with advanced disease.